REMARKS

Claims 9-27 are in this application. Claims 9-11 have been withdrawn. Claims 12, 13, 14, 19, and 27 have been amended to delete the phrase "or a polynucleotide that encodes CT-1." Claims 16, 18, and 24 have also been amended. Claims 1-8 and 20-26 are cancelled. Claims 2-27 are being cancelled because of the amendment deleting the phrase "or a polynucleotide that encodes CT-1" from claim 12 and 19.

According to the Examiner, claims 12-19 and 27 are objected to an encompassing subject matter withdrawn from consideration in view of applicants' election of the invention of Group II. In view of the amendment of claims 12, 13, 14, 19, 22 and 27, it is respectfully requested that this objection be withdrawn.

The Examiner states that claims 12-17 are not enabled. This is respectfully traversed.

There are a variety of deleterious stimuli leading to liver damage, liver failure and death. During surgical hepatectomy or liver transplantation for example, the liver is subjected to periods of ischemia followed by reperfusion. During ischemia the lack of oxygen causes mitochondrial dysfunction. On reperfusion the dysfunctional mitochondria produce big amounts of oxygen free radicals that cause marked oxidative stress and cell damage resulting in hepatocyte apoptosis (cell death) and large areas of necrosis. The liver injury produced by ischemia/reperfusion during liver resection or transplantation importantly compromises the ability of the liver to regenerate. Thus, after partial hepatectomy the ischemia/reperfusion damage of the residual liver lobules prevents an adequate regenerative response and this regenerative failure may cause liver failure during the postoperative period, leading frequently to death of the patient. Consequently, the use of hepatoprotective cytokines to defend the liver against damage will favor the regenerative response thus reducing the risk of hepatic failure after liver resection of transplantation. The same occurs when other damaging stimuli (viral, metabolic, immunologic or toxic) cause liver injury. In the cases

of acute, subacute or fulminant hepatitis the use of hepatoprotective cytokines not only reduces liver cell damage but also would facilitate the regenerative response and the full recovery of liver function. Similar concepts could be applied for cases of chronic liver damage (chronic hepatitis and liver cirrhosis).

Therefore, CT-1 administration will act on the liver by protecting against hepatic damage and, as a result, by stimulating hepatic regeneration. Both effects are important and occur simultaneously or sequentially. CT-1 administration lessens liver damage as the main effect, and this, in turn, will facilitate hepatic regeneration.

CT-1 is for prophylactic use, for example, administration of CT-1 before surgery -or exposure to hepatotoxins- to defend the liver against injury, and for therapeutic use of CT-1, for example, administration of CT-1 to reduce existing liver damage.

An important indication of CT-1 is the administration of CT-1 to patients subjected to surgical hepatectomy or liver transplantation. Prophylactic administration of CT-1 before surgical intervention will prepare the liver cells for better resisting ischemia/reperfusion injury that will occur during and after surgery, thus favoring subsequent regeneration. In this setting CT-1 prevents liver damage and consequently stimulates regeneration. Support for these indications are found on example 10 . One method for achieving this effect is the use of CT-1 as a component of the preservation medium for the transplanted liver.

Similarly, it is intended that CT-1 might be given to a subject who has ingested a hepatotoxic substance even before there is any alteration of liver function tests.

CT-1 can be administered to patients with acute liver injury such as patients with acute, subacute or fulminant hepatitis of any origin (viral, autoimmune, alcoholic or drug-induced) hepatitis. In these cases CT-1 will lessen liver damage thus helping to maintain liver function and stimulating regeneration. Similar beneficial effects are seen for chronic liver diseases (such as chronic hepatitis, cirrhosis or cancer).

The claimed methods are supported and enabled as disclosed in the specification and based

on common knowledge.

The experimental work disclosed in the description of the present application, by the way of the examples and the figures present in the specification and with regard to the common knowledge existing in the field at the priority date, provide solid evidence that those examples themselves constitute experimental models well known in the prior art and commonly and widely employed to assess the therapeutic methods claimed in the present application and thus, it is clear that the claims are enabled.

The hepatoprotective effect conferred by CT-1 is the result of protecting hepatocytes from apoptosis and necrosis leading to cell death, the effect necessitated because of exposure to, for example, toxic agents, viral infections, alcohol consumption, graft refusal, autoimmune responses, etc. Due to the protective effect of CT-1, hepatocyte survival and proliferation achieve hepatic regeneration of the damaged liver.

Experimental model of Concanavalin A (ConA)-induced acute liver damage in male Balb/c mice 830 g of weight) (Examples 10 and 14b; figures 6A and 7). This model resembles the pathology of human autoimmune hepatitis (1) and **is an accepted model of liver damage caused by pro-inflammatory cytokines as occurs in chronic viral hepatitis, chronic autoimmune hepatitis and also in fulminant, sub-acute and acute hepatitis of various etiologies.** Con-A-induced hepatitis is an experimental model of T-cell dependent liver injury in which the viability of the hepatocytes is compromised by the high amounts of hepatotoxic cytokines released from T lymphocytes and Kupffer cells. Tiegs, G., Hentschel, J., Wendel A. A T-cell dependent experimental liver injury in mice inducible by concanavalin-A. J. Clin. Invest. 1992. 90: 196-203. Both, apoptotic and necrotic hepatocyte cell death are evidenced in this model of acute liver injury. Tiegs, G., Hentschel, J., Wendel A. A T-cell dependent experimental liver injury in mice inducible by concanavalin-A. J. Clin. Invest. 1992. 90: 196-203. Lasarte, J.J., Sarobe, P., Boya, P., Casares, N., Arribillaga, L., Lóópez-Dííaz de Cerio, A., Gorraiz, M., Borráás-Cuesta, F., Prieto, J. A recombinant adenovirus encoding hepatitis C virus core and E1 proteins protects mice against cytokine-induced liver damage. Hepatology 2003. 37: 461-470.

Treatment of mice with an adenovirus harboring CT-1 cDNA (AdCT-1, 10^7 pfu) 48 h before ConA (100mg/Kg, intravenous), significantly prevented the rise in serum transaminases (ALT) observed in those mice that received the control adenovirus (AdLac-Z). TUNEL staining of liver sections obtained from these mice evidenced a significant protection from apoptosis only in mice that were treated with AdCT-1. Histological examination of liver tissue from control and AdCT-1-treated mice, further confirmed the protective effect of CT-1. Those mice that received AdCT-1 were virtually devoid of any histological signs of necrosis and apoptosis.

Experimental model of acute liver failure after subtotal (85%) liver resection in Fischer rats (Example 9). **This is a highly reproducible model of acute liver failure with very high mortality (near 100% of the animals), and a good test for drugs and therapeutic manouevres with hepatoprotective potential against subacute or fulminant hepatitis.** Kobayashi, N., Fujiwara, T., Westerman, K.A., Inoue, Y., Sakaguchi, M., Noguchi, H., Miyazaki, M., Cai, J., Tanaka, N., Fox, I.J., Leboulch, P. Prevention of acute liver failure in rats with reversibly immortalized human hepatocytes. Science 2000. 287. 1258-1262. In this model, the remanent liver tissue undergoes liver cell death, ultimately resulting in compromised liver function leading to death. Kobayashi, N., Fujiwara, T., Westerman, K.A., Inoue, Y., Sakaguchi, M., Noguchi, H., Miyazaki, M., Cai, J., Tanaka, N., Fox, I.J., Leboulch, P. Prevention of acute liver failure in rats with reversibly immortalized human hepatocytes. Science 2000. 287. 1258-1262. Panis, Y., McMullan, D.M., Emond, J.C. Progressive necrosis after hepatectomy and the pathophysiology of liver failure after massive resection. Surgery 1997. 121: 142-149. Both necrotic and apoptotic hepatocyte cell death have been observed in this experimental model . Panis, Y., McMullan, D.M., Emond, J.C. Progressive necrosis after hepatectomy and the pathophysiology of liver failure after massive resection. Surgery 1997. 121: 142-149.

Intravenous administration of AdCT-1 (10^8 pfu) to rats 48 h prior to subtotal liver resection significantly improved survival, as compared with mice that were administered the control adenovirus (AdLac-Z, 10^8 pfu) (Fig. 5). The enhanced survival afforded by AdCT-1 was accompanied by the activation of survival pathways that oppose apoptotic and necrotic cell death,

such as STAT3, Akt and ErK1/2 activation. Indeed in rats that received AdCT-1 therapy, the remanent following subtotal hepatectomy was protected against apoptosis and necrosis and maintained a good viability and could undergo regeneration as result of the hepatoprotective action of CT-1. In this case CT-1 behaves both as an hepatoprotective agent and as an stimulant of liver regeneration after extensive liver resection.

Experimental model of acute liver damage induced by D-galactosamine (D-Gal) plus TNFalpha administration to mice (Example 10 and figure 6C). In this model hepatocytes are sensitized by D-Gal to the pro-apoptotic effects of TNFalpha, **mimicking the clinical manifestations of a variety of immunologically mediated liver diseases.** Tiegs, G., Niehöörster, M., Wendel, A. Leukocyte alterations do not account for hepatitis induced by endotoxin or TNFa in galactosamine-sensitized mice. Biochem. Pharmacol. 1990. 40: 1317-1322.

Male Balb/c mice (30 g of weight) were treated with D-Gal (25mg/mouse, intraperitoneal) and TNFalpha (0.5 ug/mouse, intravenous). Intravenous administration of AdCT-1 (10^7 pfu) to mice, 48 h prior to D-Gal plus TNFalpha treatment, resulted in significant protection from liver injury as evidenced by reduced serum transaminases levels and prevention of TUNEL staining, as compared with control mice that received the AdLac-Z adenovirus (10^7 pfu) (Fig. 6C). **These observations further support the hepatoprotective capacity of CT-1 in liver disease of autoimmune (e.g., chronic active hepatitis) or viral origin (chronic viral hepatitis)**, in which TNFalpha plays a central role. Hussain, M., Mustafa, A., Gallati, H., Mowat, A., Mieli-Vergani, G., Vergani, D. Cellular expression of tumour necrosis factor-alpha and interferon-gamma in the liver biopsies of children with chronic liver disease. J. Hepatol. 1994. 21: 816-821.Spengler, U., Zachoval, R., Gallati, H., Jung, M., Hoffmann, R., Riethmuller, G., Pape, G. Serum levels and in situ expression of TNF-alpha and TNF-alpha binding proteins in inflammatory liver diseases. Cytokine 1996. 8: 864-872. Sheron, N., Lau, J., Daniels, H., Goka, J., Eddleston, A., Alexander, G., Williams, R. Increased production of tumor necrosis factor alpha in chronic hepatitis B virus infection. J. Hepatol. 1991. 12:241-245.

Experimental model of acute liver injury mediated by Fas stimulation (Example 10 and

figure 6B). Hepatocytes express Fas antigen constitutively, and the liver is very sensitive to Fas-mediated hepatocellular death. Kondo, T., Suda, T., Fukuyama, H., Adachi, M., Nagata, S. Essential roles of the Fas ligand in the development of hepatitis. Nat. Meddicine. 1997. 3:409-413. Moreover, **Fas has been shown to be important in human liver injury triggered by different pathological conditions, such as chronic alcohol consumption, viral hepatitis, cholestatic liver disease and graft-versus-host disease .** Yoon, J-H., Gores, G.J. Death receptor-mediated apoptosis and the liver. J. Hepatol. 2002. 37:400-410. Administration of anti-Fas agonistic antibody to mice is a well-characterized experimental model that mimics part of the pathogenic mechanisms of the above-metioned conditions. The type of hepatocellular death after Fas ligation includes both apoptosis and necrosis. Haga, S., Terui, K., Zhang, H.Q., Enosawa, S., Ogawa, W., Inoue, H., Okuyama, T., Takeda, K., Akira, S., Ogino, T., Irani, K., Ozaki, M. Stat-3 protects against Fas-induced liver injury by redox-dependent and-independent mechanisms. J. clin. Invest. 2003. 112:989-998. Male Balb/c mice (30 g of weight) were treated with either AdCT-1 (10^7 pfu) or the control AdLac-Z adenovirus (10^7 pfu). After 48 h, mice received a single intravenous injection of 1.5 ug of anti-Fas antibody (Jo2). Evaluation of hepatocellular damage by assessment of serum transaminases, histological examination of tissue sections, and TUNEL staining, clearly indicated that those mice that received the AdCT-1 adenovirus were protected form hepatocellular damage (Fig. 6B). **In this sense Ct-1 may serve to treat any kind of acute liver damage in which Fas ligation is involved such as severe alcoholic, viral or autoimmune hepatitis.**

In summary, the test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosure in the patent coupled with information known in the art without and to do so without undue experimentation The fact that some experimentation is required does not condemn the specification nor does the fact that the skilled worker may have need to consult outside references to produce an operable result. When considering the enablement test, the person who must be able to utilize the specification to make or use the invention is one "skilled in the art to which the invention pertains or with which it is most nearly connected" and so a reasonable degree of expertise can be assumed. The specification includes a description to enable a person skilled in the art to make and use the invention as claimed. It is cleared that the requirement of 35 U.S.C. 112 , first paragraph

is met.

According to the Examiner, claims 12, 14, 20 and 22 are rejected under 35 USC 102(b) as being anticipated by Jin et al., (1996) Cytokine, Vol.8 (12):920-926. This rejection is respectfully traversed.

As stated by the Court of Appeals for the Federal Circuit in **Structural Rubber Products Co. v. Park Rubber** 749 F.2d 707 223 USPQ 1264 (Fed Cir 1984) , in order to anticipate, the single prior art reference must disclose each and every element of the claimed invention. Jin et al. does not do this.

According to the abstract in the Jin et al. reference, "CT-1 can induce cardiac hypertrophy in vivo...It stimulated the growth of the liver, kidney, and spleen..."However, no where in Jin et al. is in disclosed that the growth in the liver was due to hepatic regeneration or a hepatic protective effect. On page 921 of Jin et al. it is stated that liver weight increased in animals treated with the high dose of CT-1. However, there is no histological data or other information that discloses the reason for the increase in the liver weight or that the increase in weight is due to growth of the liver or an increase in the number or protection of hepatocytes in a liver. It is respectfully stated that the Examiner's statement that Jin et al. teach administration of cardiotrophin-1 to stimulate liver growth is not based on the data provided in Jin et al. because there is no disclosure that states nor suggests that the increase in liver weight is due to growth of the liver, hepatic regeneration, prevention of damage or treatment of an intrahepatic tumor.

Therefore, as each and every element of the claimed invention is not disclosed in Jin et al., it is respectfully requested that the rejection be withdrawn.

It is submitted that the present application is in condition for allowance and favorable consideration is respectfully requested.

Respectfully submitted,

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